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# CARNIVOROUS PLANT NEWSLETTER

Journal of the International Carnivorous Plant Society

Volume 36, No. 3 September 2007

# CARNIVOROUS PLANT NEWSLETTER

**Journal of the International Carnivorous Plant Society**
**www.carnivorousplants.org**

**Volume 36, Number 3**
**September 2007**

Front Cover: *Nepenthes* 'Lady Pauline,' upper pitcher. Photograph courtesy of Borneo Exotics. Article on page 68.

Back Cover: The new cultivar *Dionaea* 'Bohemian Garnet'. Photograph by Miroslav Srba. See article on page 68.

Carnivorous Plant Newsletter is dedicated to spreading knowledge and news related to carnivorous plants. Reader contributions are essential for this mission to be successful. Do not hesitate to contact the editors with information about your plants, conservation projects, field trips, or noteworthy events. Contributors should review the "Instructions to Authors" printed in the March issue of each year. Advertisers should contact the editors.Views expressed in this publication are those of the authors, not the editorial staff.

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ICPS, Inc.
PMB 322
1564-A Fitzgerald Drive
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Editors:
Barry A. Rice, P.O. Box 72741, Davis, CA 95617, USA, email: barry@carnivorousplants.org
Jan Schlauer, Zwischenstr. 11, D-60594 Frankfurt, Germany, email: jan@carnivorousplants.org
Page Layout: Steve Baker, email: steve@carnivorousplants.org

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John Brittnacher, Manager • john@carnivorousplants.org

# New Cultivars

Keywords: cultivar: *Dionaea muscipula* 'Bohemian Garnet', *Nepenthes talangensis* × *maxima* 'Lady Pauline'.

## *Dionaea muscipula* 'Bohemian Garnet'

Submitted: 15 January 2007

This plant was obtained from an F2 cross between an all red clone (*Dionaea* 'Royal Red') and a more typically colored *Dionaea* 'Sawtooth' plant in 2000. It does not demonstrate any new mutation in general leaf form, but it does have new attributes which distinguish it from other, previously existing *Dionaea* cultivars. Establishing this plant name as a new cultivar will help keep proper order in the wide assortment of *Dionaea muscipula* cultivars already registered.

This new cultivar differs in its growth from other *Dionaea* cultivars in the following ways:
1)All-red coloration of plants, similar to *Dionaea* 'Akai Ryu'.
2)Marginal trichomes are reduced and divided to numerous toothlets similar to *Dionaea* 'Sawtooth'. Note that the trichomes on *Dionaea* 'Red Piranha' are dentate, but not sawtooth.
3)Adult plants are about 4-6 cm in diameter, only about half the size of most *Dionaea* (including *Dionaea* 'Sawtooth').
4)Enormous proliferation. One individual is able to produce 10-30 or more offshoots during a single growth season.

I coined the cultivar name, 'Bohemian Garnet', on 17 November 2006. This name reflects the all-red color of plants, their small jewel-like size, and fact that they are usually seen in large number—such as several garnets are often used in a single piece of jewelry. Finally, the name also honors the Czech Republic (Bohemia), the country of origin for this cultivar. In Czech, the name can be written *Dionaea* 'Český Granát'.

Cultivation of *Dionaea* 'Bohemian Garnet' is standard, no increased sensitivity to fungi or any other pests was observed. I recommend the continual removing of its numerous offshoots to obtain as large a plant as possible. These offshoots root well even though they are very small. *Dionaea* 'Bohemian Garnet' must be propagated vegetatively to maintain all the described characters of this unique clone. Propagation via seeds is not necessary because of extreme proliferation.

Because of horticultural trade that occurred before the registration of this cultivar name, specimens of this cultivar are already in a large number of European collections. Therefore I ask all growers who obtained their plants from my collection to change the labels of any plants called "red sawtooth" to the new established name *Dionaea* 'Bohemian Garnet'. Contact me if you want me to validate the identity of your plant, if you are unsure if it is *Dionaea* 'Bohemian Garnet'.

Specimens of this plant can be obtained directly from me. More photographs of the plant are available at my web site: http://www.sarracenia.cz/informace/dionaea/bohemiangarnet/index.html

—Miroslav Srba • Želivského 1970 • 430 01 Chomutov • Czech Republic • srba@sarracenia.cz

## *Nepenthes talangensis* × *maxima* 'Lady Pauline'

Submitted: 12 October 2006

This outstanding artificial hybrid between *N. talangensis* and *N. maxima* originates from a sterile culture I obtained 1998 from Thomas Alt, Germany. It is believed that the cross was made either by him or by one of his close associates in Germany.

For a number of years, plants of this hybrid parentage, all of similar overall appearance and morphology were being circulated erroneously as "*N. bongso* × *maxima*." One of the reasons for the name confusion was probably due to the fact that at that time, *N. talangensis* had not yet been formally described.

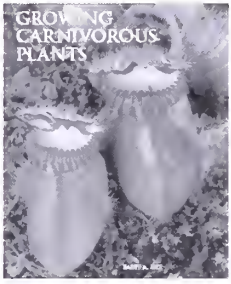
Figure 1: *Nepenthes* 'Lady Pauline' lower pitchers. Photograph courtesy of Borneo Exotics.

In general morphology as well as pitcher shape this plant is intermediate between the parent species. The lower pitchers are similar to *N. maxima* in coloration, being richly speckled in dark purple, with a wide dark purple peristome up to 3 cm across. The pitchers are distinctively shorter and wider than those of *N. maxima*, reaching dimensions of 14 cm by 8 cm. They are widest just beneath the peristome. Also, the pitcher mouth is more horizontal and round than that of *N. maxima*. The upper pitchers are similar to the lower pitchers in overall size, but are more infundibular (funnel-shaped) and are lighter in coloration, being translucent pale cream with red stripes and red peristome. The leaf blades are linear-lanceolate, obtuse at the base. The leaf blade apex is obtuse, occasionally somewhat emarginate in mature specimens.

In order to preserve the characteristics of this plant, it should be propagated vegetatively.

This plant is named in honour of Dame Pauline Norma Harris D.L., mother of Martin Harris, Commercial Director of Carpetright plc, who has very generously sponsored Borneo Exotics for the Chelsea Flower Show in 2006. Martin Harris coined the name on 15 March, 2006.

—Robert Cantley • 262 B/5 Millagahawatta • Hokandara Road • Thalawathugoda • Sri Lanka • nepenthes@borneoexotics.com




## Growing Carnivorous Plants (the book)

Yes, you can get copies from the publisher or Amazon.com, but buy your copy directly from the author, Barry Rice, and he will sign it for you! Very cool, indeed!

Instructions at http://www.sarracenia.com/cp.html, or send a check (payable to "Barry Rice" in US$) to: P.O. Box 72741, Davis CA 95617, USA. Total cost: US$47.95 within the USA. International customers–refer to the web site.

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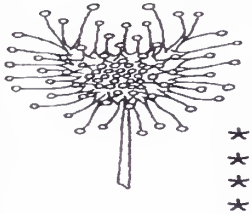

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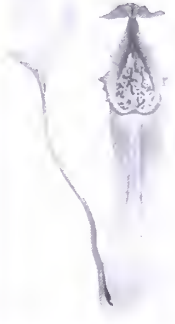

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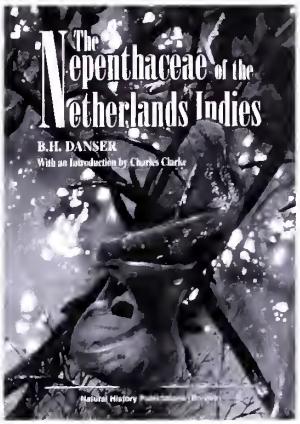



Danser, B.H. 2006. The Nepenthaceae of the Netherlands Indies (first published in 1928, as Contributions A L'etude De La Flores Des Indes Neerlandaises. XV). Natural History Publications (Borneo), Kota Kinabalu 221 pp., 36 black and white figures, ISBN 983-812-115-0. 18.5 × 25.5cm (harcover). US$41, shipping extra.

Reviewed by Barry Rice

All casual and serious students of *Nepenthes* are familiar with Danser's essential 1928 publication, in which he discussed about 65 species in great detail, and most with quite lovely black and white line drawings. Unfortunately, the work (often just referred to as "Danser") has been out of print for so long that most of us have had to satisfy ourselves with self-made photocopies (or photocopies of copies of copies...) kept in three-ring binders.

While this might be a suitable solution for many old publications, Danser is different. Yes, this book is dated. But Danser was so important in summarizing our understanding of the genus that current works usually use it as a launch point for further discussions. Having a high quality copy of the work is very desirable so you can easily cross reference its contents with subsequent *Nepenthes* monographs. The line drawings are far more nicely presented than can be obtained from photocopied or web versions.

To add further delight to the pleasure, the famous *Nepenthes* expert Dr. Charles Clarke helps lead this voyage of adventure on the high seas of *Nepenthes* history, by prefacing the work with a fifteen page discussion of Danser—both the man and the eponymous work. With Clarke as your Admiral at the helm, Danser is more approachable than ever before.

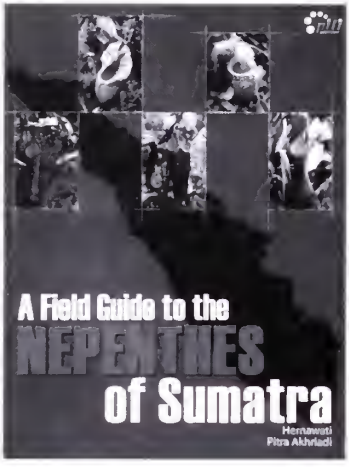
Unfortunate omissions from this book are further important works by Danser, specifically his 1935 and 1940 publications (both in Bull. Jard. Bot. Buitenzorg) in which he described *N. spathulata* and *N. densiflora*, respectively, and elucidated his opinions on tricky matters such as *N. pilosa*. We will need to keep these in our three-ring binders! However, the new version of Danser (1928) is still a valuable addition to our ever-enlarging collections of carnivorous plant books.

The work can be purchased from the publisher, at http://www.nhpborneo.com.

## 7th Conference of the ICPS in 2008!

It gives me great pleasure to announce that the 2008 International Carnivorous Plant Society Conference will be held in Sydney, Australia. The venue chosen is the Royal Botanic Gardens, in the heart of the city, close to many facilities. Since this is the first time this biennial conference has been held in the Southern Hemisphere it was deemed appropriate for it to be held at a time when the carnivorous plants across most of southern Australia would be at their best, and so the conference will be held from the 25nd to the 28th of September. The conference will include a number of lectures and demonstrations, and there will be some optional field trips to the Blue Mountains and Mt Tomah Botanic Gardens, both within 3 hours drive from Sydney. Provisions are also being made for a field trip to see *Cephalotus*, pygmy sundews and tuberous sundews in South Western Australia for conference attendees able to make it to this part of the country during their stay down under. Stay tuned for further details, and please mark the dates in your diaries; we'd love to see you in Sydney in 2008.

Greg Bourke




Hernawati, and Akhriadi, Pitra. 2006. A field guide to the *Nepenthes* of Sumatra. PILI-NGO Movement, Bogor, West Java, Indonesia. ISBN 979-3143-18-5. xiv+94 pp. 15 cm × 20 cm. Price to be announced.

Reviewed by Charles Clarke

A field Guide to the *Nepenthes* of Sumatra by Hernawati and Pitra Akhriadi is exactly as the name suggests—a short book presenting basic information on thirty-one Sumatran taxa, the majority of which were surveyed in the wild by the authors over a period of several years, as part of a research program funded by British Petroleum (BP). The upshot of that work was a report on the conservation status of Sumatran *Nepenthes* species, based on the authors' field observations. As part of their work, the authors felt that it would be good to increase the public profile of *Nepenthes* in Sumatra by publishing a guide of this type; this is the result. The guide is a soft cover production printed on very thin paper with colour photographs throughout, along with several line drawings. The opening sections introduce the reader to the island of Sumatra and the morphology of *Nepenthes*. The main part of the book provides a brief description and photos of each species, as well as providing several spot characters and information on the typical habitat, IUCN conservation status, geographical and altitudinal range, etc. The final section deals with the conservation status of Sumatran *Nepenthes*. Much of this information is available in other publications, but the key difference here is that it was collected by local Indonesian workers, who have direct access to many of these species on a regular basis.

It's hard to strike a balance reviewing books of this type—on one hand, it is important to be critical and impartial in appraising the work, but when you've known the authors for years and were involved in various stages of their applications for funding, field work and report writing, as I have, there has to be some element of bias and I admit to that freely. Readers who speak English as their first language will find many minor grammatical errors in the text, but I have no issue with this, as I could not achieve a result of similar quality if I had to write in Indonesian, and their efforts in producing a document that is generally very readable is to be commended. The authors are acutely aware of the problems associated with the illegal collection of *Nepenthes* from the wild, and have been careful not to give away any important locations. The correct identification of species throughout is refreshing, but to be expected as the authors know the local taxa well and have read all the major works on the genus published in recent years.

Where possible, the authors have provided their own photographs, but have also used (with our permission) photos by myself and Ch'ien Lee and we are very grateful for their acknowledgement of this (many books published in SE Asia "borrow" images from photographers without obtaining permission, a blatant infringement of copyright). The quality of the paper, printing and images is perhaps a little low, but given the very meagre funds available to produce this book, this is understandable. In summary, this book will be of interest to anyone who collects publications on *Nepenthes*, or those who are interested in the conservation status of *Nepenthes* or carnivorous plants in general. I like it a lot and commend the authors for their efforts.

## Looking Back: CPN 25 years ago

The September 1982 issue was dominated by three articles on *Nepenthes* in Malaysia, Singapore, and cultivation. Interest in the genus has only increased in the intervening 25 years, as a conference and exhibition on *Nepenthes* was just held at Sarawak, Malaysia. Let's hope interest (and wild populations of *Nepenthes*) persist for another twenty-five years!

# Variations in the Content and Isozymic Composition of Nepenthesin in the Pitcher Fluids Among *Nepenthes* Species

Kenji Takahashi • Laboratory of Molecular Biochemistry • School of Life Sciences, Tokyo University of Pharmacy and Life Sciences • 1432-1 Horinouchi, Hachioji, Tokyo 192-0392 • Japan • kenjitak@ls.toyaku.ac.jp
Masao Tanji • Department of Biophysics and Biochemistry • Graduate School of Science • The University of Tokyo, Bunkyo-ku, Tokyo 113-0033 • Japan
Chiaki Shibata • Department of Biology • The Nippon Dental University • Chiyoda-ku, Tokyo 102-8159 • Japan

Keywords: carnivory: *Nepenthes* — chemistry: *Nepenthes*.

Received: 18 June 2006

## Introduction

Carnivorous plants are of great evolutionary interest in their function to catch prey (mainly insects) to digest their proteins as a nitrogen source (Juniper *et al.* 1989; Frazier 2000). Among them, *Nepenthes* species utilize pitchers which contain digestive fluid with the acid proteinase nepenthesin (Amagase *et al.* 1969; Woessner 2004). Previous inhibitor studies (Lobareva *et al.* 1973; Takahashi *et al.* 1974; Tökés *et al.* 1974) indicated that nepenthesin belongs to the aspartic proteinase family. Recently, two nepenthesins have been purified to homogeneity and characterized from *N. distillatoria*, and their primary structures were elucidated by cloning of the cDNAs from the pitcher of *N. gracilis* (Athauda *et al.* 1998, 2002, 2004; Takahashi *et al.* 2005). Cloning of aspartic proteinases from the pitcher tissue of *N. alata* has also been reported recently (Ann *et al.* 2002a); however, these enzymes appear to belong to vacuolar aspartic proteinases. The peptide bond cleavage specificity of nepenthesin has been investigated using *Nepenthes* pitcher fluid (Ann *et al.* 2002b), partially purified nepenthesin (Amagase *et al.* 1969; Tökés *et al.* 1974) and fully purified nepenthesin (Athauda *et al.* 2004; Takahashi *et al.* 2005). The results of these investigations appear to be rather variable; this may be partly due to the differences in the substrates and *Nepenthes* species used and enzyme purity. *Nepenthes* is known to include around 70 different species (Juniper *et al.* 1989), and so far it is not clear how much quantities and how many kinds of such proteinases are generally present in the pitcher fluids of various *Nepenthes* species and how much they are different from each other in structural and enzymatic properties. The present study was performed as a preliminary attempt to shed some light on these issues. First, we assayed the acid proteinase activity in the pitcher fluids of various *Nepenthes* species to obtain information on the enzyme contents among them. Second, we analyzed the isozymic composition by using native polyacrylamide gel electrophoresis combined with activity staining. The results indicate that there are significant variations in the content of nepenthesin in the pitcher fluids among *Nepenthes* species but that the proteolytic activity is largely due to two groups of nepenthesins.

## Materials and Methods

The *Nepenthes* species and hybrids examined were grown as free as possible from the access of insects, and pitcher fluids were collected within a few days before and after the lid opening. The proteinase activity was determined at pH 2.0 using bovine hemoglobin as a substrate as described (Athauda *et al.* 1989), and expressed in pepsin equivalent using porcine

pepsin (Sigma) as a standard. The pH 2.0 was chosen to compare the activity with that of porcine pepsin obtained under the standard pepsin assay conditions; most workers agree on a single pH optimum for *Nepenthes* secretion around 2.2, very similar to that of pepsin (Juniper *et al.* 1989). Electrophoresis and activity staining were performed as follows. An appropriate portion (20µl) of each pitcher fluid was submitted to native polyacrylamide gel electrophoresis using 7.5% acrylamide gel and Tris-glycine buffer, pH 8.7, and then proteinase activity was examined by activity staining with hemoglobin as a substrate at pH 1.7 and 7.3 as described (Furihata *et al.* 1972). The pH value of each pitcher fluid was measured using a glass electrode in a Horiba pH-meter.

## Results and Discussion

Table 1 shows the pH values and the acid proteinase activity of the pitcher fluids of 17 different *Nepenthes* species and hybrids examined. The pH values varied from 2.6 to 4.7, and the proteolytic activity per ml of the pitcher fluid ranged from 0 to 52µg as expressed in pepsin equivalents.. Thus, there are marked variations in the content of the acid proteinase among the species examined. The total proteolytic activity in the pitcher fluid varied from 0 to 105 µg, partly reflecting the differences in pitcher size. It is notable that the open pitcher fluids always contained a substantial level of acid proteinase activity whereas no activity was detected with at least three of the ten samples of the unopen pitchers examined. These results suggest that before the lid opening, in at least some cases, the acid fluid is secreted first into the pitcher, followed later by the secretion of the enzyme.

Figure 1 shows the results of activity staining after native polyacrylamide gel electrophoresis of the pitcher fluids from 15 different *Nepenthes* species. When the activity was

Table 1: Acid proteinase activities in the pitcher fluids of various *Nepenthes* species

| No. | *Nepenthes* species | No. of pitchers used[a] | pH | Activity[b] per ml | Activity[b] per pitcher |
|---|---|---|---|---|---|
| 1 | *N.* × *mixta* | 1 (o) | 4.7 | 7.5 | 52 |
| 2 | *N.* × *coccinea* | 6 (u/o) | 4.4 | 6.9 | 7 |
| 3 | *N.* × *superba* | 1 (u) | 4.3 | 8.9 | 83 |
| | | 2 (u/o) | 4.7 | 9.3 | 33 |
| 4 | *N. thorelii* | 3 (u) | 2.8 | 52 | 36 |
| | | 4 (o) | 4.3 | 35 | 62 |
| 5 | *N. rafflesiana* | 1 (u) | 3.9 | 0 | 0 |
| 6 | *N. alata* × *merrilliana* | 2 (u) | 4.3 | 0 | 0 |
| 7 | *N.* hybrid[c] | 1 (o) | 3.9 | 1.0 | 17 |
| 8 | *N. mirabilis* | 1 (u) | 3.7 | 0 | 0 |
| | | 2 (o) | 2.8 | 5.1 | 28 |
| 9 | *N.* × *internedia* | 1 (u) | 4.1 | 0.4 | 1 |
| 10 | *N. thorelii* × *N.* × *coccinea* | 1 (u) | 3.8 | 1.6 | 10 |
| | | 1 (o) | 2.6 | 9.1 | 101 |
| 11 | *N. dicksoniana* × *dyeriana* | 3 (u) | 4.4 | 14 | 35 |
| 12 | *N.* × *henryana* | 1 (u) | 3.8 | 0.8 | 2 |
| 13 | *N. alata* | 2 (o) | 4.1 | 3.0 | 6 |
| 14 | *N. maxima* | 1 (u) | 3.9 | 26 | 24 |
| 15 | *N.* × *wrigleyana* | 1 (o) | 3.8 | 6.7 | 27 |
| 16 | *N. ventricosa* | 2 (o) | 4.4 | 41 | 43 |
| 17 | *N. thorelii* × *maxima* | 1 (o) | 4.2 | 28 | 105 |

[a]For those samples where two or more pitchers were used, the pitcher fluids were combined, then used for the pH and activity measurements. The states of pitchers are shown in parentheses: u, unopen pitcher; o, open pitcher; u/o, a mixture of unopen and open pitchers.

[b]Activity was determined with hemoglobin as a substrate at pH 2.0 and denoted in terms of µg of porcine pepsin equivalents.

[c]A hybrid with disputed parentage.

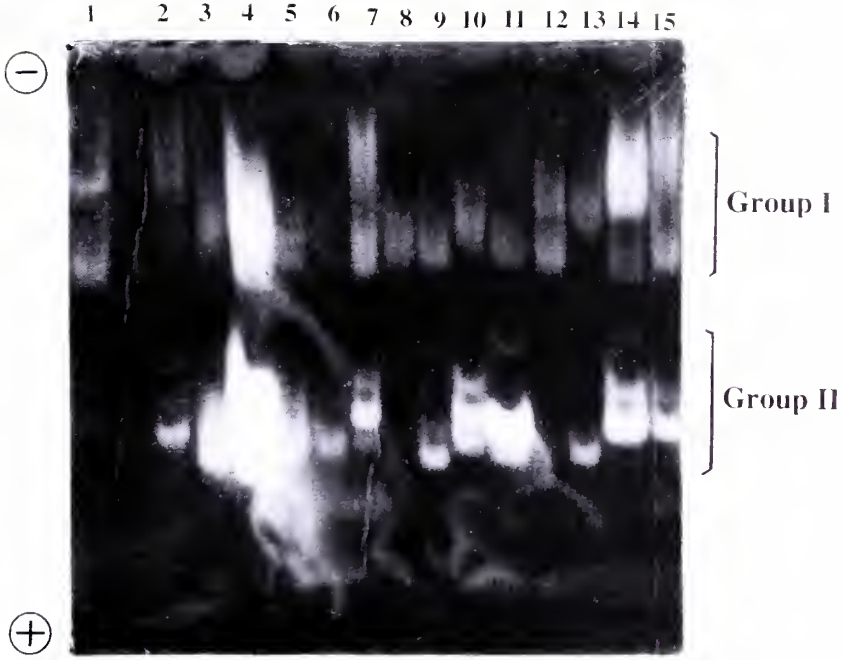



Figure 1: Activity staining of acid proteinases from the pitchers of *Nepenthes* spp. A portion (20μl) of each pitcher fluid was submitted to native polyacrylamide gel electrophoresis at pH 8.7, and the proteinase activity was stained by incubation of the gels with hemoglobin as a substrate at pH 1.7. The sample numbers correspond to those in Table 1. Pitcher fluids from unopen pitchers were used for sample Nos. 3-6, 8-12 and 14. The pitcher fluids of sample Nos. 16 and 17 were not analyzed.

stained at pH 1.7 (Figure 1), the extents of staining were roughly comparable with the activity per each sample used. It is notable that activity was detected on the gel even with sample Nos. 5, 6 and 8 which showed practically no activity in the hemoglobin assay. This contradiction may be due to the difference in sensitivity of the two methods; however, the possibility that the bands obtained with sample Nos. 5, 6 and 8 may be due to artifacts cannot be completely excluded. Almost all species examined gave two or occasionally up to four acid proteinase bands, which seem to constitute two major groups (groups I and II) notably different in electrophoretic mobility, and hence in isoelectric point. Each sample showed one to two bands in the group I region and one to three bands in the group II region. This multiplicity is thought to reflect the isozymic variations of nepenthesin. On the other hand, when the activity staining was performed at pH 7.3, no proteinase activity was detected (data not shown), indicating that the pitchers may contain no endopeptidase capable of digesting hemoglobin other than the acid proteinase nepenthesin. A definite conclusion on this point, however, can be made only after a more detailed research.

Occurrence of the two groups of acid proteinases is consistent with the fact that two distinct types of acid proteinases with different isoelectric points (nepenthesins I and II) were found in and isolated and characterized from the pitcher fluids of *N. distillatoria* (Athauda *et al.* 1998, 2002, 2004; Takahashi *et al.* 2005), and that the corresponding two enzymes were structurally characterized by cloning and sequencing of the two cDNAs from the pitcher tissue of *N. gracilis* (Athauda *et al.* 2004; Takahashi *et al.* 2005). The isoelectric points of nepenthesins I and II from *N. gracilis* were calculated to be 3.94 and 3.09, respectively. Therefore, nepenthesins I and II are thought to correspond to the groups I and II acid proteinases, respectively. As for nepenthesin I from *N. gracilis*, two isozymic forms, nepenthesin Ia and Ib, were found, which have slight differences in amino acid sequence. Two isozymic forms with slight differences in amino acid sequence were also found in both nepenthesins I and II from *N. distillatoria*. Nepenthesin I is a

glycoprotein (Athauda *et al.* 1998), and therefore the multiple bands in the group I proteinases (see Figure 1) may be partly due to the heterogeneity in the carbohydrate moieties.

Although this study is a preliminary one using a limited number of *Nepenthes* samples, the results indicate that there is a significant variation in the content of the proteolytic activity in the pitcher fluid among the *Nepenthes* species, but that the proteolytic activity is largely due to the two groups of enzymes, nepenthesins I and II. The variations in the enzyme content may be in part related to the phylogenetic and habitat diversities of the species (Meimberg *et al.* 2001).

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# FLAVONOIDS OCCURRING IN THE STICKY RESIN ON *RORIDULA DENTATA* AND *RORIDULA GORGONIAS* (RORIDULACEAE)

ECKHARD WOLLENWEBER • Institut fuer Botanik der TU Darmstadt • Schnittspahnstrasse 3 • D-64287 Darmstadt • Germany • wollenweber@bio.tu-darmstadt.de

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## Introduction

The leaves of *Roridula dentata* L. and *Roridula gorgonias* Planch., two South African plant species, bear numerous glandular hairs, with droplets of sticky liquid at their tips. The plants' habitus thus largely resembles that of many *Drosera* species, and also *Drosophyllum lusitanicum* (L.) Link (Droseraceae)[1]. The resemblance is further indicated by the fact that *Roridula* plants trap insects very successfully. Flower morphology, however, led botanists to place *Roridula* in a separate family, Roridulaceae. In the scope of ongoing research on structures and distribution of flavonoid aglycones in plant exudates (see e.g. Wollenweber *et al.* 2005), the insect-trapping glue of *Roridula dentata* and *Roridula gorgonias* has now been studied for the presence of flavonoids. Flavonoids are phenolic compounds with a basic three ring C15-skeleton, that can display numerous variations, depending on the state of C-ring oxydation, number and position of hydroxyl and methoxyl groups and other substituents (see Figure 1 for examples). Natural products of this type are ubiquitous throughout the plant kingdom in glycosidic form, i.e., they are linked with sugar molecules and are therefore water soluble and located within the plant tissue. The occurrence of sugar-free molecules, called aglycones, on the other hand, is restricted and

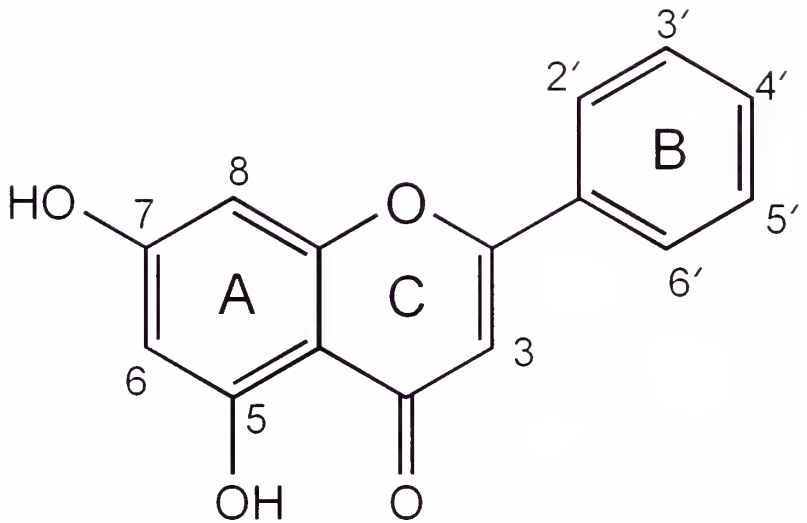


Figure 1: Flavones: 5,7,4'-OH=Apigenin; 5,7,3',4'-OH=Luteolin; 5,7,3',4',5'-OH=Tricetin. Flavonols: 3,5,7,4'-OH=Kaempferol; 3,5,7,3',4'-OH=Quercetin.

[1]According to recent molecular studies, *Drosophyllum* is more closely related to Dioncophyllaceae/Ancistrocladaceae and should be excluded from Droseraceae (J. Schlauer, pers. comm.).

appears to be correlated with the presence of secretory structures and the formation of lipophilic exudates (leaf resins, leaf waxes, essential oils). The detection and identification of flavonoid aglycones in the mucilage of *Roridula* is reported here.

## Experimental

One dried plant of *Roridula gorgonias* was obtained from the Botanischer Garten der TU Darmstadt, but most of the material of *R. dentata* and *R. gorgonias* was kindly supplied by three people, cultivating these plants and breeding the obligately associated carnivorous hemipteran insect, *Pameridea roridulae* as a hobby (see Acknowledgements section). Seeds were from different origin. The flavonoid patterns observed in different samples of each species exhibited the same flavonoid pattern and were, therefore, combined for analysis. Voucher specimens are kept in the herbarium of the Botanischer Garten Darmstadt.

On rinsing with acetone, 106 g of dried old *Roridula gorgonias* leaves yielded 15.3 g of sticky resinous exudate. This material was "defatted", by solution in a small volume of hot MeOH, cooling to -10° C, and removal of precipitated material by centrifugation. The supernatant was evaporated to yield 13.1 g of residue. Ca. 3.6 g of material remained as a mushy glue, insoluble even in boiling MeOH. The soluble portion was chromatographed on Sephadex LH-20 (Pharmacia) eluted with methanol to separate flavonoids from the predominant terpenoids. Fractions were monitored by thin layer chromatograpy (tlc) on silica, and those containing flavonoids were combined. The flavonoid mixture was further purified by preparative TLC on silica plates (SIL G 25, Macherey-Nagel), developed with toluene/MeCOEt 9:1 (v/v). Flavonoid bands were marked under UV light and scraped off. Elution of the silica with ethanol yielded individual flavonoids. Fractions were monitored and comparison with markers was achieved by TLC on polyamide (DC 11, Macherey-Nagel) with the following solvents:
i) PE 100-140/toluene/MeCOEt/MeOH 12:6:1:1 (v/v/v/v);
ii) toluene/PE100-140/ MeCOEt /MeOH 12:6:2:1 (v/v/v/v);
iii) toluene-dioxane-MeOH 8:1:1 (v/v/v), and on silica with solvent;
iv) toluene/MeCOEt 9:1 (v/v).

Chromatograms were viewed under UV (366 nm) before and after spraying with Natural Product Reagent A (0.2 % of diphenyl-boric acid 2-aminoethyl ester in MeOH). One of the isolated flavonoids was further characterized by its mass spectrum (Varian MAT 212 spectrometer at 70 eV) and by its $^{1}H$ NMR and $^{13}C$ NMR spectra (DMSO-$d_6$, Brucker ARX 400 spectrometer at 400 MHz and 100 MHz, respectively). $^{1}H$ NMR: $\delta$ =6.98 (s, H-3), 6.40 (d, J = 2.0 Hz, H-6), 6.78 (d, J = 2.0 Hz, H-8), 7.19 (d, J = 2.0 Hz, H-2'), 7.22 (d, J = 2.0 Hz, H-6'), 12.88 (s, 5-OH), 9.73 (s, 5'-OH), 3.77 (s, OMe), 3.88 (s, OMe), 3.90 (s, OMe). $^{13}C$ NMR: 163.4 (C-2), 104.8 (C-3), 181.9 (C-4), 161.0 (C-5), 98.0 (C-6), 165.2 (C-7), 92.6 (C-8), 157.2 (C-9), 104.7 (C-10), 125.6 (C-1'), 104.8 (C-2'), 153.5 (C-3'), 139.7 (C-4'), 150.9 (C-5'), 107.8 (C-6'), 56.0 (7-OMe), 56.1 (3'-OMe), 60.0 (4'-OMe). These data are in accordance with those reported for tricetin-7,3',4'-trimethyl ether (Zahir *et al.* 1996).

In the case of *Roridula dentata*, only a small amount of leaf material was available. The exudate was recovered and further processed as described above. The flavonoid portion obtained after column chromatography on Sephadex LH-20 was immediately analyzed by comparative TLC with markers.

## Results and Discussion

With one exception, the flavonoids were readily and unambiguously identified by direct TLC comparisons with authentic markers available in the author's lab. In the exudate of *R. gorgonias*, apigenin and its 4'-monomethyl ether (acacetin) and 7,4'-dimethyl ether, luteolin 7,3'-dimethyl ether (velutin) and luteolin 7,3',4'-trimethyl ether, and the 3',4'-dimethyl (apometzgerin), 3',4',5'-trimethyl and 7,3',4',5'-tetramethyl ether (corymbosin) of tricetin were thus

identified. For structural formulas see Figure 1. Based on its molecular mass (M+ at m/z 344, indicating a flavone with two OH and three OMe groups) and on extensive experience with the thin layer chomatographic behaviour of flavonoid spots as related to chemical structures, one further flavone was assumed to be the 7,3',4'-trimethyl ether of tricetin. Its identity was proven by its NMR spectra (see "Experimental" section).

The apigenin and luteolin derivatives present in the resinous exudate of *R. gorgonias* are widespread flavones whereas the tricetin derivatives, on the other hand, are rare plant constituents. Tricetin 3',4'-dimethyl ether, first reported as a *C*-glycoside in a liverwort (Theodor *et al.* 1980), has been found only three times previously as an aglycone (c.f. Valant-Vetschera & Wollenweber 2006). Tricetin 7,3',4'-trimethyl ether has been reported only once before, from *Lethedon tannensis* Spreng (Thymelaeaceae; Zahir *et al.* 1996, as *L. tannaensis* Forst.). The 3',4',5'-trimethyl ether, first reported from several Poaceae (Kaneta & Sugiyama 1973), was later found on the leaf surface of *Nonea pulla* (Boraginaceae; Wollenweber *et al.* 2002) and in the exudate of *Asarina procumbens* (Scrophulariaceae; Wollenweber unpublished). Tricetin 7,3',4',5'-tetramethyl ether, originally reported from *Webera corymbosa* (Rubiaceae; Joshi & Rane 1967), was found recently on *Centaurea incana* (Asteraceae) and on *Walsura piscidia* (Meliaceae) (for references, see Valant-Vetschera & Wollenweber 2006).

In the exudate of *R. dentata*, kaempferol 3,4'-dimethyl ether and traces of kaempferol 3-methyl ether, quercetin 3,3',4'-trimethyl ether and traces of quercetin 3,3'-dimethyl ether were identified. Two minor components remain thus far unidentified, due to lack of material. The four flavonols found are widespread compounds.

It is striking that the trapping gland exudate of *R. gorgonias* contains exclusively flavones, whereas in *R. dentata* it contains flavonols only. It should also be mentioned that, in both species, no difference was detected between the flavonoid profiles of fresh leaves on the one hand, and of wilted or dried leaves on the other hand.

As was already mentioned in the Introduction, classical taxonomic characters led botanists to segregate Roridulaceae from Droseraceae. The different character of the insect-catching exudates has been used to support the distinctness of these two families (cf. Marloth 1925). However, to the best of our knowledge the chemical composition of *Roridula* exudate has not yet been studied. The insect-trapping mucilage of *Drosera* and *Drosophyllum* is an aqueous solution of acidic polyasaccharides, and hence hydrophilic. The exudate of *Roridula*, on the other hand, is lipophilic material, consisting primarily of triterpenoids, natural products that are often found as major components of plant resins. As shown in this paper, the exudate also contains very small amounts of flavonoids. Analysis of the exudate terpenoids, which make up the bulk of the extremely sticky resin secreted by stalked glands on *Roridula* leaves, is in progress and will be published in the future.

Acknowledgements: Thanks are due to Stefan Schneckenburger (Darmstadt) who drew my interest to the exudate of *Roridula*, as well as to Stefan Ippenberger (Feichten), Klaus Keller (Augsburg) and Martin Reiner (Bayerbach) who kindly supplied leaf material of *Roridula dentata* and *R. gorgonias*, respectively. I also wish to thank James N. Roitman (Albany, CA) for NMR analysis of tricetin 7,3',4'-trimethyl ether and critical review of the English text.

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## Literature Reviews

By Doug Darnowski

Reut M.S., and Fineran, B.A. 2000. Ecology and vegetative morphology of the carnivorous plant *Utricularia dichotoma* (Lentibulariaceae) in New Zealand. New Zealand J. of Bot. 38: 433-450.

The authors examined eight sites spread across New Zealand to better understand the ecology of *Utricularia dichotoma*, defined broadly to include *U. monanthos* and *U. novae-zealandiae*. Essentially they found that *U. dichotoma* prefers open, nutrient-poor, brightly lit, wet sites and could often be associated with a few species of plants, especially various bryophytes and *Juncus articulatus*. It could, however, extend its range into other habitats once well established, such as permanently flooded areas, but not without limits—not surprisingly, *U. dichotoma* was never found growing in calcareous soils. Those trying to grow *U. dichotoma* or any other members of its Australia/New Zealand species complex will find these data helpful, and this paper also presents some valuable insights into the plasticity of one widespread group of terrestrial bladderworts. (DWD)

Sanabria-Aranda L., Gonzalez-Bermudez A., Torres N.N., Guisande C., Manjarres-Hernandez A., Voloyes-Valois V., Diaz-Olarte J., Andrade-Sossa C., and Duque S. R. 2006. Predation by the tropical plant *Utricularia foliosa*. Fresh. Biol. 51: 1999-2008.

In this paper, Sanabria-Aranda *et al.* examined prey capture by the large, pan-American *Utricularia foliosa* for the importance of bladder size, the type of prey captured, and the strategies used for trapping. This is a species which can be found as far north as Florida, easily identified by its flattened stems. In this paper, the work was done in Colombian Amazon. Increasing trap sizes did not provide disproportionate benefits in terms of nutrients obtained from prey, and the prey trapped most commonly were those types whose behavior made them most likely to contact the traps and the trap hairs, similar to what has been found by several other studies. Perhaps most interesting and novel was the finding that longer trap hairs/"antennae" led to significantly improved trapping rates, confirming in the field previous experimental studies. (DWD)

# A Case of *Nepenthes rafflesiana* With a Lotus-like Flower!

Jorge Joel Torres-Rivera • 324 Tejas Street • URB. San Gerardo • San Juan, PR 00926 • USA • jardin_emilia@hotmail.com

Keywords: ecology: cultivation: *Nepenthes rafflesiana* – observations: *Nepenthes rafflesiana*.

The first *Nepenthes* I ever grew was a specimen of *N. rafflesiana* that I bought in 2003 from a local nursery in Puerto Rico. This plant did well, and encouraged me to grow more carnivorous plants. My collection of *Nepenthes* and other carnivorous plants grew. Many of my *Nepenthes* started to produce flowers, but despite my luck with these other plants, my very first-ever *Nepenthes* purchase refused to flower. It produced many pitchers with increasingly large size, and its leaves were huge. It was very healthy in my outdoor growing house, which has no walls but a roof that helps filter sunlight. Exposed to the climate, the plant loves the humid and warm weather of Puerto Rico. Yet, it did not flower!

Finally, in the spring of 2006 my beloved *N. rafflesiana* did the magic. I noticed a protuberance at the base of the growing point, which finally developed into an inflorescence. However, even at an early stage I could see the inflorescence was different from the ones I had seen on my other *Nepenthes* plants; it was bigger and fatter than normal. As it enlarged, I saw it did not consist of many little flowers in a crowded panicle, but was instead a group of sepals enclosing a single fat and lonely flower. By no means it was similar to the typical *Nepenthes* inflorescence!

When the sepals opened it revealed a single huge stamen shaped like a dark wine-colored mushroom, surrounded by more than a dozen sepals. To me it looked more a flower from a lotus (*Nuphar* sp.) than that of a *Nepenthes*! The stamen produced pollen, but I did not have a female plant in flower to test if it was viable. After several days the pollen faded, but the flower stayed apparently fresh.

I wondered—were all the flowers from this plant going to be so strangely abnormal? I could only wait for the next inflorescence to see! It was not until the autumn of the same year that the plant finally produced a new inflorescence. It was a normal male inflorescence. As I write this (in late December 2006), this inflorescence is starting to fade, but the original lotus-flower still looks as fresh as when it first opened!

Now I am waiting for the next flowering of this plant. Will the flower be normal, or will it be another oddity? And what caused the strange flower in the first place? An interesting mystery!

If you want to see other images of my lotus-flowering *Nepenthes rafflesiana*, you can visit my tropical and exotic plants nursery eBay web site (Emilia's Garden; http://stores.ebay.com/EMILIAS-GARDEN).

Figure 1: Different views of the *Nepenthes rafflesiana* plant, its normal inflorescence, and the strange lotus-like abnormal flower.

# What is the evidence for medicinal value of carnivorous plants?

Michael M. Chan[1,2], Mallory M. Chan[3], Edward D. Chan[4,5] • K613E, Goodman Building • National Jewish Medical and Research Center • 1400 Jackson Street • Denver, CO 80206 • USA • chane@njc.org

[1]George Washington High School
[2]Denver Botanic Gardens
[3]University of Colorado at Boulder
[4]National Jewish Medical and Research Center
[5]Division of Pulmonary Sciences and Critical Care Medicine, University of Colorado School of Medicine, Denver, Colorado

Keywords: praetical uses: *Dionaea muscipula*, *Drosera*, *Nepenthes khasiana*, *Pinguicula*, *Sarracenia purpurea*.

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## Introduction

Throughout history, plants have been used for medicinal purposes. Many of the modern allopathic medicines used today are either directly or indirectly derived from plants. Examples include the cardiotonic and antiarrhythmic agent digoxin (derived from the foxglove—*Digitalis purpurea*), antimalarial quinine (derived from the bark of the cinehona tree—*Cinchona officinalis*), the mydriatic (pupil dilatory) agent belladonna (derived from the nightshade *Atropa belladonna*), aspirin (derived from the bark of the willow tree—*Salix alba*, etc.), taxol (derived from the yew tree—*Taxus brevifolia*) and vincristine (derived from the periwinkle—*Catharanthus roseus* (=*Vinca rosea*)). Although carnivorous plants have been used medicinally, to date not a single compound derived from carnivorous plants has been widely-accepted in modern allopathic medicine. *Pinguicula* spp., *Dionaea muscipula*, *Drosera* spp., *Sarracenia purpurea*, and *Nepenthes* spp. are the carnivorous plants frequently touted as having therapeutic potential. We herein review the existing literature on the medical uses of these plants.

## Individual Plants

The butterwort (*Pinguicula*) has been used as folk medicine in Scandinavia, where farmers have applied its leaves on open wounds of cattle (Alm 2005); it has been surmised that an antiseptic in the plant helps heal wounds and prevents superinfection (D'Amato 1998). In Norway, *Pinguicula vulgaris* is used to remove lice and to treat ringworms (Alm 2005). In Britain and Ireland, the juice from the leaves of *P. vulgaris* has also been used to treat chapped skin conditions (Allen & Hatfield 2004). In Wales, a "syrup" of *P. vulgaris* was used to induce vomiting or bowel movements. The syrup was also combined with butter to produce an ointment touted to cure "liver obstructions."

Extracts of the Venus flytrap (*Dionaea muscipula*) have been marketed as "Dionaea Tonic" and as "Carnivora" (www.800herbal.com, accessed July 2005). Dionaea Tonic is claimed by the vendors to contain a proteolytic enzyme that dissolves abnormally growing tissue. The commercial sale or production of Carnivora in the USA, which is marketed as an anticarcinogen, is prohibited by the Food and Drug Administration (FDA) (USDA 1992). Carnivora is distributed by a German physician who has sold over 100 other drugs that he claimed, without much evidence, can treat a variety of diseases including heart diseases and multiple sclerosis (USDA 1994). In addition, the drugs are seldom labeled in English despite the intent to target U.S. citizens; all

have received an automatic detention (i.e., are banned) from the FDA. The FDA has gone to great lengths to prohibit the sale of Carnivora in the U.S., including warnings to the doctor, to the German shippers of the drugs, and to promoters of these drugs.

The fact that the sticky secretions of sundews (*Drosera* spp.) do not dry out in the sun has led some to believe that the plant is effective against debilitating diseases (Millspaugh 1974). The round-leafed sundew (*Drosera rotundifolia*) has been touted to treat a number of pulmonary diseases including tuberculosis (Millspaugh 1974). As noted by the 16th century herbalist-physician John Gerarde in American Medicinal Plants: "The later physitians have thought this herbe to be a rare and singular remedie for all those that be in a consumption of the lungs" (Millspaugh 1974). Gerarde asserted that these claims were in fact based on the erroneous conclusion that because sundews are resistant to dessication, this translated to their ability to protect against consumptive diseases. Nevertheless, extracts of the plant have been used in "pulmonary ulcerations," asthma, and to "destroy warts and corns" (Millspaugh 1974). Practitioners of homeopathy have recommended sundew extracts to treat cough from asthma, tuberculosis, and other pulmonary disorders (Millspaugh 1974). In Ireland, extracts of the plant were used as a remedy for asthma and leaves boiled in milk were used for whooping cough (Allen & Hatfield 2004). Research studies indicate that *D. rotundifolia* and *D. madagascariensis* have anti-inflammatory effects (Paper *et al.* 2005). Ethanol extracts and aqueous extracts were tested using the HET-CAM assay (Hen's Egg Test—Chorioallantic Membrane; a test used to measure level of inflammation). Both extracts of *D. rotundifolia* and the ethanol extract of *D. madagascariensis* were found to inhibit inflammation.

Native Americans used the roots of *Sarracenia purpurea* to treat smallpox (Millspaugh 1974). F. H. Bignell claimed that the plant saved his brother from smallpox and left him without the usual scarring (Millspaugh 1974). Dr. John Thomas Lane was skeptical of the drug, which contains an alcohol extract of *Sarracenia* roots. He stated that he lost more than 50% of his patients using the drug (Millspaugh 1974). In reality, there is no evidence to indicate the drug is effective against smallpox. One study found that chloroform extracts of *S. flava* had antitumor activity against lymphocytic leukemia (Miles & Kokpol 1976).

Currently, an extract of *S. purpurea* is the principal ingredient in the drug Sarapin® (High Chemical, Levittown, PA). This extract is produced by distillation of the entire *S. purpurea* plant. Because Sarapin® was introduced prior to the drug laws of the 1960s, it does not have to be reviewed by the FDA unless there is a change in manufacturing, labeling, route of administration, or indications. Although its exact mechanism of action is not known, Sarapin® is claimed to have analgesic properties and to relieve neuralgic pain, sciatic pain, intercostal neuralgia, alcoholic neuritis, occipital neuritis, brachial plexus neuralgia, meralgia paresthetica, lumbar neuralgia, coccydynia, and trigeminal neuralgia (www.sarapin.com). In severe myofascial trigger pain syndrome, injection of a local anesthetic, calcium, and Sarapin® into trigger points was claimed to desensitize and deactivate rapid-firing pain fibers. Sarapin® was the main ingredient used to treat acute frontal cephalalgia (headache caused by dilated cerebral arteries) (McCalla 1995) and omohyoideus myofascial pain syndrome, a chronic pain condition in which there is tenderness of the omohyoideus muscle (a neck muscle) (Rask 1984). To determine the efficacy of Sarapin® in entrapment neuropathy of the foot, 75 patients were randomized to one of three treatment regimens (Rosen 2004). The first group was given a combination of lidocaine, Sarapin®, Vitamin B-12, and dexamethasone phosphate. The second was given lidocaine, Sarapin®, and Vitamin B-12. The third was given lidocaine, bupivocaine, and Vitamin B-12. Although the study concluded that the addition of Sarapin® relieved pain, the study was flawed because proper control conditions were not met. Another study tested the therapeutic value of Sarapin® in lower back pain (Manchikanti *et al.* 2000). In this study, a group of 180 patients was divided into three groups. The first group was treated with local anesthetic. The second group was given the addition of Sarapin® while the third was given a corticosteroid and Sarapin®. Sarapin® was found to relieve pain more than local anesthetic alone. In a study of horses, Sarapin® lacked any anesthetic efficacy in the abaxial sesamoid block model (Harkins *et al.* 1997).

*Nepenthes* species have several ethnocultural uses in Southeast Asia (personal communications, P. D'Amato). Medicinally, the roots of *Nepenthes* have been used to regulate menstruation and to reduce fever (D'Amato 1998). The sterile fluid of an unopened pitcher is said to relieve

asthma, to have antibacterial properties, and is used as an eyewash and antiseptic (D'Amato 1998).

## Antimicrobial effects of carnivorous plants

Many modern and well established antibiotics have originally been isolated from various fungi, higher orders of bacteria, or are synthetic agents modified from the original natural compounds. To the best of our knowledge, there is no antibiotic in allopathic medicine that is derived from plants. The first manufactured antibiotic—penicillin—was isolated from the mold *Penicillium chrysogenum*, and streptomycin, the first effective drug against the causative agent of tuberculosis, was derived from the soil organism *Streptomyces*.

In certain regions of Southeast Asia, the fluid of *Nepenthes* has been used as an antiseptic. People in England, Ireland, and Scandinavia have used *P. vulgaris* extract as an antibiotic on wounds. Despite claims that carnivorous plants have antibacterial properties, apparently only one study has systematically evaluated this assertion Wu (1995, unpublished data). This unpublished and unrefereed investigation concluded that *Drosera*, *Sarracenia*, *Pinguicula*, and *Nepenthes* have efficacy against drug-resistant strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Mycobacterium smegmatis*. Wu (1995, unpublished data) obtained extracts of carnivorous plants using ethanol, methanol, hexane, or water as solvents. He tested the antimicrobial activity of the carnivorous plants by exposing bacteria grown on Mueller-Hinton agar to their extract using the disk diffusion susceptibility method. He found that the extracts of *Nepenthes khasiana* exhibited exceptional antibacterial activity against many of the bacteria tested. Although plumbagin and quercetin are the two compounds said to be the active antimicrobial agents in many carnivorous plants Wu (1995, unpublished data), the concentrations of plumbagin and quercetin in *N. khasiana* did not correlate with its antibacterial effectiveness. Using mass spectroscopy and several chromatographic techniques to separate the plant extracts, a third organic compound, yet to be identified, was considered to be the antibacterial agent of *N. khasiana* Wu (1995, unpublished data).

Other studies have found *Drosera* species to have antimicrobial activities. Ferreira *et al.* (2004) investigated the antimicrobial activity of extracts of Brazilian *Drosera* (*D. communis*, *D. montana* var. *montana*, *D. brevifolia*, *D. villosa* var. *graomogolensis*, and *D. villosa* var. *villosa*) against a number of bacteria. Didry *et al.* (1998) investigated the antimicrobial properties of the tuberous sundew *D. peltata*. and found that a chloroform extract of *D. peltata* exhibited antimicrobial activity, found to be due to plumbagin.

## Discussion

Claims and commercialization have been made of the medicinal properties of carnivorous plants. Nearly all are based on anecdotal case reports or testimonies. Companies that manufacture "tonics and tinctures" from carnivorous plants rely on old folk medicine and rumors of the plants' medicinal properties. In short, there are insufficient data to support claims that carnivorous plants have medicinal properties. Only by conducting rigorous, properly controlled studies, can it be definitively established whether they have any therapeutic efficacy with acceptable toxicity. If found to be ineffective, these findings will likely help in the conservation of the dwindling stands by decreasing their unwarranted harvestation. If certain compounds derived from carnivorous plants are found to be promising in *in vitro* studies, then double-blind, randomized, placebo-controlled clinical studies will ultimately be needed to determine their true medical values.

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# Differentiation of *Utricularia ochroleuca* and *U. stygia* populations in Třeboň basin, Czech Republic, on the basis of quadrifid glands

Bartosz J. Płachno • Institute of Botany • Department of Plant Cytology and Embryology • Jagiellonian University • Grodzka 52 • PL-31-044 Cracow • Poland • bartek78pl@poczta.onet.pl
Lubomír Adamec • Institute of Botany • Academy of Sciences of the Czech Republic • Section of Plant Ecology • Dukelská 135 • CZ-379 82 Třeboň • Czech Republic • adamec@butbn.cas.cz

Keywords: physiology, taxonomy: *Utricularia ochroleuca*, *U. stygia*.

## Introduction

*Utricularia ochroleuca* R. Hartm. is an amphibious/aquatic carnivorous plant occurring relatively rarely throughout Europe and North America in peat bogs and shallow standing dystrophic waters (Taylor 1989; Schlosser 2003). This species rarely flowers, is sterile, and is possibly of hybridogenic origin (i.e. *U. minor* × *U. intermedia*; Thor 1988). Thor (1988) split this taxon into two species, *U. ochroleuca* s. str. and *U. stygia* Thor (see Figure 1), on the basis of minor differences in corolla morphology, the number of leaf teeth tipped with bristles, and, especially, in the structural details of the quadrifid (i.e. four-armed) ×-shaped glands (hairs) in their carnivorous traps. The rarity of flowering, the unreliability of the tooth number on the leaves as a determination marker, preventing from easy determination (the numbers greatly overlap; Thor 1988; Schlosser 2003), and the rarity of these two species in Central Europe contributed partly to the neglect or refusal of the Thor's concept of *U. stygia* as a separate species (in the Czech Republic e.g., Holub & Procházka 2000; Adamec & Lev 2002; Sirová *et al.* 2003). The strongest argument that *U. ochroleuca* s. str. and *U. stygia* are separate species hinges upon differences in their quadrifid glands. According to Thor (1988), *U. ochroleuca* s. str. has a very wide angle between the shorter arms of the glands (usually 146-197°, on average 171°, 2 SD=51°), while in *U. stygia*, the angle is much narrower (usually 52-97°, on average 74°, 2 SD 45°). Quadrifid glands (hairs) in *Utricularia* traps are known to be associated with carnivory. They secrete digestive enzymes and absorb released nutrients (e.g., Juniper *et al.* 1989; Sirová *et al.* 2003). Recently, cell wall ingrowths typical of "transfer cells" have been reported in *U. intermedia* quadrifid glands (Płachno & Jankun 2004) demonstrating an intensive short-distance solute transport between the glands and the trap fluid.

However, in the last 5-10 years, *U. stygia* has been more widely accepted as a taxon distinguishable from *U. ochroleuca* s. str. Populations have been identified in several European countries in addition to Thor's (1988) original distribution in Scandinavian countries, Denmark, Scotland (Hackney 2000) and Germany. Zidorn (1996) found it in Austria (Tirolia), Tassara (2002) in N Italy, Wolff (1993) in E Germany, and Schlosser (2003) demonstrated its presence in SW Germany. It also occurs in NE Russia (Karelia) and France (Michaux 2002; Schlosser, pers. comm.). Additional discoveries are likely, and it can be expected that *U. stygia* has an even broader European spread than we currently know. In the Czech Republic, *U. stygia* has never been reported though it may occur there (Adamec & Lev 2002). *Utricularia ochroleuca* s. lat. is a critically endangered species of the Czech flora (with only about fifteen sites) and the centre of its Czech distribution lies dominantly in Třeboň basin, S Bohemia (Holub & Procházka 2000). In the 1990s, the number of its existing sites in this region was about twelve but some of these sites no longer exist (Adamec, unpubl.).

The aim of this study was to explore the occurrences and character variabilities in *U. ochroleuca* s. str. and *U. stygia* by studying *U. ochroleuca* s. lat. plants from all known existing sites (in 2003) in Třeboň basin, Czech Republic, by measuring size and geometric parameters of the quadrifid glands arms, and in this way, to look for *U. stygia*. The map of recent distribution of *U. ochroleuca* s. str. and *U. stygia* in the Czech Republic is also presented.

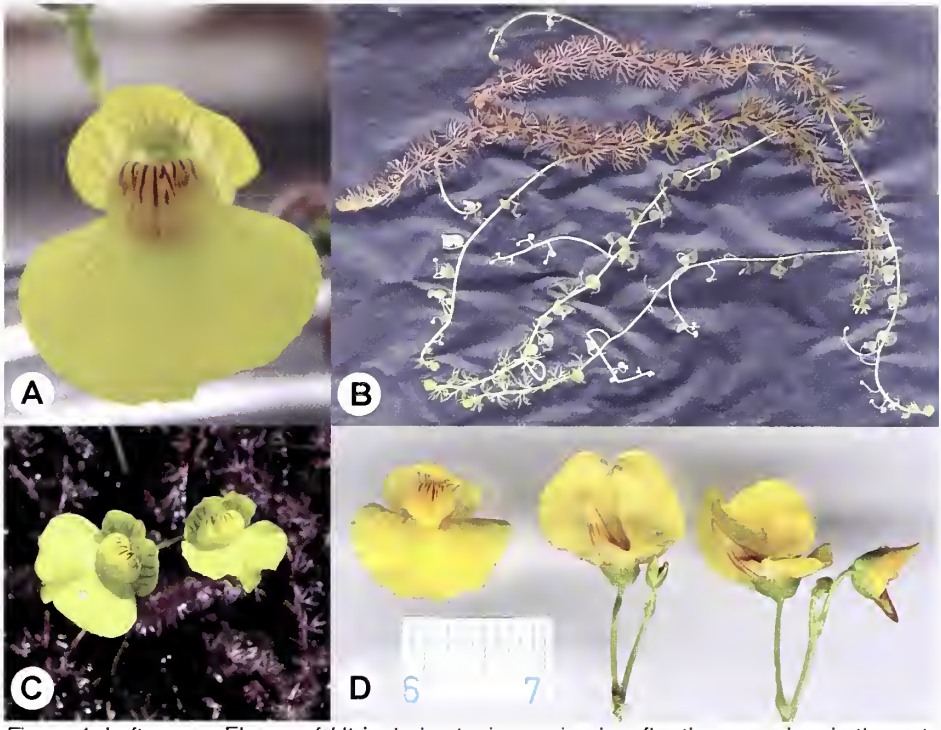


Figure 1: Left upper: Flower of *Utricularia stygia* growing in a floating aquarium in the outdoor culture of the Institute of Botany at Třeboň, Czech Republic; May 2005. The plants originate from Švarcenberk. Right upper: Scanned shoots of *U. stygia* from Hliníř; May 2000. Left bottom: Flowering *U. stygia* in a very dense stand at Hliníř; June 2003. Photo by J. Flísek. Right bottom: Scanned flower of *U. stygia* from the outdoor culture; August 2003. The plants originate from Švarcenberk. Note reddish tinge of the corolla and relatively long spur. The other images by L. Adamec.

## Material and methods

On 6 August 2003, plants of *U. ochroleuca* s. lat. were collected from all previously known sites in the Třeboň Basin Biosphere Reserve and Protected Landscape Area, S Bohemia, Czech Republic (approx. 49 N, 14°45'E; 425-450 m a.s.l.). The eight sites were in Ponědrážka, Hliníř, Švarcenberk, Ptačí blato, Rod, Naděje, Vizír, and Podsedek. The habitats were peat bogs, usually in the littorals of eutrophic fishponds (Adamec & Lev 2002). Plants of *U. ochroleuca* s. lat. were found neither at Černiční fishpond nor in Františkov village (floodplain of the Dračice river). At Hliníř, the plants were collected from two different bog pools (proximal and distal) which were isolated by a 200 m wide band of forest. Shoot segments (usually colourless and leafless) of four to five different plants with mature traps were collected from each site and placed in small plastic vials. Analyses of quadrifid glands were performed one day after the plant collection. At Ponědrážka site, the peaty soil was almost dry and the plants were strikingly short, with only a few traps formed. In this case, the bladders of one plant were analysed while the other four plants were transferred to a small aquarium in an outdoor culture in the Institute of Botany at Třeboň. After mature traps had been formed in these plants the analysis of glands was performed on 8 September 2003.

One mature trap from each of four collected plants from each site was used for microscopic investigation of the glands. Quadrifid glands in longitudinally halved traps in water were photographed using a computer-controlled microscope camera Hitachi HV-20C (Hitachi Denshi. Ltd., Tokyo, Japan; software Lucia; Imaging Industry, Prague) at 200 magnification. Within each trap, relatively representative glands were imaged from an undamaged (usually central) part of

| Site | Long arms | | | Short arms | | | Length ratio | Angle (°) | |
|---|---|---|---|---|---|---|---|---|---|
| | L (µm) | W (µm) | L/W | L (µm) | W (µm) | L/W | Long/ Short | Long arms | Short arms |
| | *U. stygia* | | | | | | | | |
| Ponědrážka (field) | 96.5±5.5 | 10.5±0.1 | 9.15±0.44 | 74.3±3.4 | 10.3±0.1 | 7.23±0.29 | 1.30±0.17 | 35.3±5.9 | 57.1±7.7 |
| Ponědrážka (culture) | 143.3±10.1 | 11.4±0.7 | 12.6±0.47 | 94.2±6.1 | 11.4±0.9 | 8.33±0.18 | 1.52±0.03 | 44.2±3.1 | 74.0±6.7 |
| Hliníř (proximal) | 97.0±7.8 | 9.4±0.4 | 10.3±0.56 | 64.6±6.1 | 9.2±0.3 | 6.99±0.54 | 1.51±0.06 | 24.8±4.8 | 61.3±2.8 |
| Hliníř (distal) | 115.4±5.5 | 10.8±0.7 | 10.7±0.14 | 84.3±2.7 | 10.7±0.7 | 7.96±0.44 | 1.37±0.04 | 36.8±3.9 | 54.6±7.2 |
| Švarcenberk | 89.6±5.7 | 10.2±0.4 | 8.81±0.52 | 63.4±3.1 | 10.1±0.3 | 6.25±0.25 | 1.41±0.02 | 27.5±2.7 | 54.4±7.4 |
| Rod | 96.9±3.7 | 11.7±0.2 | 8.31±0.21 | 73.9±4.2 | 11.0±0.1 | 6.74±0.37 | 1.32±0.04 | 37.5±4.3 | 66.1±5.2 |
| Vizír | 102.6±4.7 | 11.4±0.1 | 9.02±0.31 | 72.3±3.4 | 10.7±0.3 | 6.74±0.20 | 1.43±0.06 | 36.5±3.9 | 66.5±9.0 |
| Total mean | 105.9±6.9 | 10.8±0.3 | 9.84±0.56 | 75.3±4.1 | 10.5±0.3 | 7.18±0.28 | 1.41±0.03 | 34.7±2.5 | 62.0±2.8 |
| | *U. ochroleuca s. str.* | | | | | | | | |
| Ptačí blato | 66.4±2.9 | 9.75±0.21 | 6.83±0.35 | 53.2±2.8 | 9.78±0.16 | 5.45±0.31 | 1.25±0.06 | 38.0±4.1 | 110.0±3.7 |
| Naděje | 79.0±5.4 | 8.37±0.16 | 9.44±0.62 | 55.2±2.9 | 8.51±0.36 | 6.49±0.23 | 1.43±0.06 | 29.0±0.6 | 134.6±9.2 |
| Podsedek | 84.6±0.8 | 9.03±0.29 | 9.41±0.35 | 66.4±1.9 | 9.01±0.34 | 7.38±0.22 | 1.28±0.05 | 36.1±4.4 | 134.7±3.5 |
| Total mean | 76.7±5.4 | 9.05±0.40 | 8.56±0.87 | 58.3±4.1 | 9.10±0.37 | 6.44±0.56 | 1.32±0.06 | 34.4±2.7 | 126.4±8.2 |
| SSD | NS | ** | NS | NS | * | NS | NS | NS | *** |

Table 1: Morphometric characteristics of quadrifid glands in traps of *U. stygia* and *U. ochroleuca* s. str. populations in Třeboň basin, S Bohemia, Czech Republic. Means of four traps collected from different plants and 1 SE intervals are shown for each population as well as total means for the both species. L, length; W, width. In each trap, sizes were measured in 10 glands and angles in 15 ones. SSD, statistically significant difference between the both species: NS, non-significant, $P > 0.05$; *, $P < 0.02$; **, $P < 0.001$; ***, $P < 0.0001$.

| Species | F P | Long arms | | | Short arms | | | Length ratio | Angle (°) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | L | W | L/W | L | W | L/W | Long/ Short | Long arms | Short arms |
| US | F | 8.07 | 3.33 | 12.9 | 6.25 | 2.19 | 4.40 | 4.50 | 3.05 | 1.14 |
| US | P | *** | * | *** | *** | NS | ** | ** | * | NS |
| UO | F | 6.83 | 9.22 | 10.7 | 7.55 | 4.53 | 14.5 | 3.04 | 1.86 | 5.38 |
| UO | P | * | ** | ** | * | * | ** | NS | NS | * |

Table 2. Statistically significant differences in measured parameters between populations within each species, as evaluated by one-way ANOVA for four individual traps from each plant population. US, *U. stygia*; UO, *U. ochroleuca* s. str.; n=7 for US; n=3 for UO. NS, non-significant, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

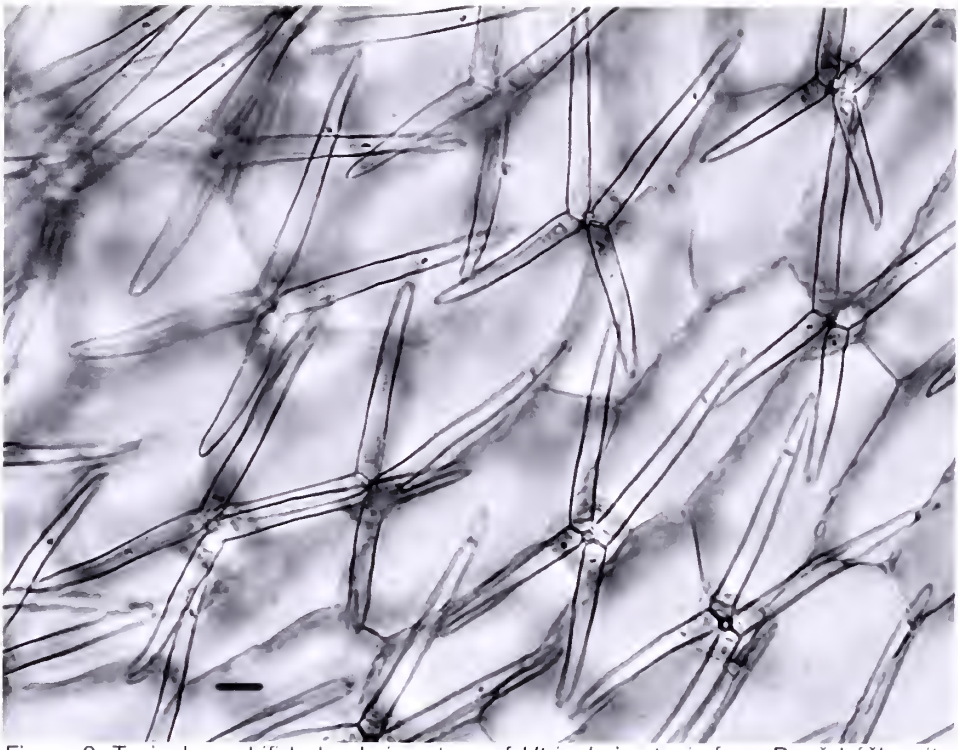

Figure 2: Typical quadrifid glands in a trap of *Utricularia stygia* from Ponědrážka site grown in outdoor culture. Black bar represents 20 μm.

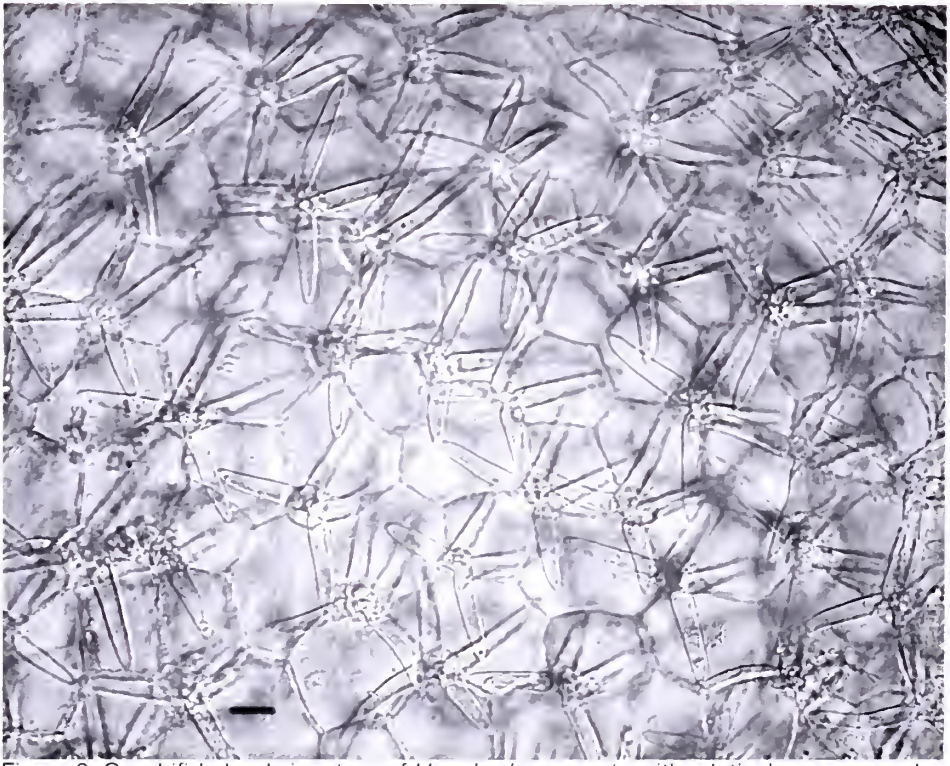

Figure 3: Quadrifid glands in a trap of *U. ochroleuca* s. str. with relatively narrow angles between the shorter arms collected from Ptačí blato site. Black bar represents 20 μm.

the trap. One image per trap was printed, and the following parameters were measured using a ruler and a protractor: length (L) and maximum width (W) of both long and short arms and angles (to the nearest 0.5 ) between the basal parts of long and also short arms (see Schlosser 2003). The sizes were measured in ten quadrifid glands (i.e., twenty values for each parameter), and angles were measured for fifteen glands. The glands that were imaged with the greatest clarity and sharpness were selected for these measurements to minimize geometric distortion of both sizes and angles. Mean values of all parameters from individual traps from each site were evaluated by one-way ANOVA (Tukey HSD test), both as within-species and between-species differences. Herbarium material of some examined specimens was deposited in the herbarium (KRA) of the Institute of Botany, the Jagiellonian University, Cracow, Poland.

## Results

When all populations of the both *Utricularia* species were taken together, a within-species statistically significant difference at least at $p<0.03$ was found in each of nine parameters estimated (Long arm L, long arm W, long arm L/W, short arm L, short arm W, short arm L/W, long arm L/short arm L, long arm angle, short arm angle; data not shown). Thus, it is reasonable to consider that the populations belong to two species. However, the size parameters of the quadrifid glands (especially lengths of long and short arms) were greatly variable even within each species, within and between populations (see Tables 1, 2), and the only statistically significant difference at $p<0.02$ between the two species was found in width of both long and short arms and in angle between the shorter arms ($p<0.0001$). Therefore, the angle between the shorter arms could be used as the only reliable criterion between the both species at a sterile state. In different populations determined as *U. stygia*, mean values of the angle ranged between 54-74 and in single traps, the mean angle never exceeded 81 . Plants from Ponědrážka, Hlinír (both proximal and distal), Švarcenberk, Rod, and Vizír were determined as *Utricularia stygia*, in contrast with the plants from Ptačí blato, Naděje, and Podsedek, which were determined as *U. ochroleuca* s. str. (see Table 1; Figures 2-4). In the latter species in different populations, the mean angles between shorter arms ranged between 110-135 and in single traps, the mean angle never fell below 101 . One-way ANOVA for individual traps revealed within-species statistically significant differences ($p<0.05$ or better) for almost all measured parameters, except for the angles between short arms in *U. stygia* (see Table 2).

## Discussion

The mean angles between the short arms of *U. stygia* from South Bohemia (Table 1; 54-74 ) are well within the range (74±22 ) given for this taxon by Thor (1988) and confirmed by Schlosser (2003). However, the mean angles between the short arms in *U. ochroleuca* s. str. from S Bohemia (Table 1; 110-135 ) are not only much lower than mean typical Scandinavian plants but even somewhat lower than a minimum angle (117 ) given by Thor (1988). But in the same paper, Thor noted that *U. ochroleuca* s. str. was a rather polymorphic species which probably might consist of some closely related taxa. In the European material, he distinguished two variants: typical one from Nordic countries, which he described in detail, and another variant which grows outside Scandinavia. This variant has smaller quadrifid glands (shorter long arms) and also a distinctly smaller angle between short arms than in the "Nordic" plants. *U. ochroleuca* s. str. from S Bohemia represent this second variant. However, in light of a great variability of morphological parameters and low number of statistically investigated world populations of *U. ochroleuca* s. lat., Thor's concept of *U. stygia* as a separate species might not be accepted. It would be also possible to accept *U. ochroleuca* s. lat. as one polymorphic species with the taxon *U. stygia* on the level of subspecies or variety. The main problem how to interpret these quantitative data on the angles for taxonomy is whether they are continuous or discontinuous on the European scale. Cytological and molecular studies (e.g., RAPD, DNA sequencing) of different populations could bring more light into this topic. Also, it might be useful to perform cross experiments between *U. intermedia* and *U. minor* and study the properties of the hybrids.

For practical determination reasons, it is possible to reliably distinguish between *U. stygia*

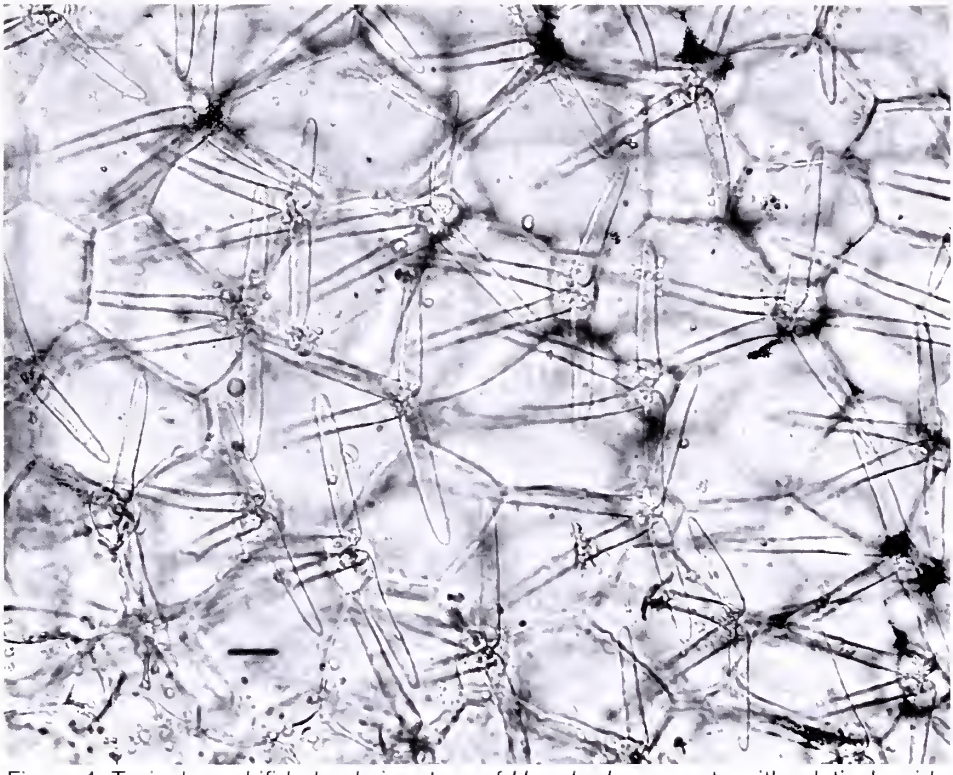

Figure 4: Typical quadrifid glands in a trap of *U. ochroleuca* s. str. with relatively wide angles between the shorter arms collected from Naděje site. Black bar represents 20 μm.

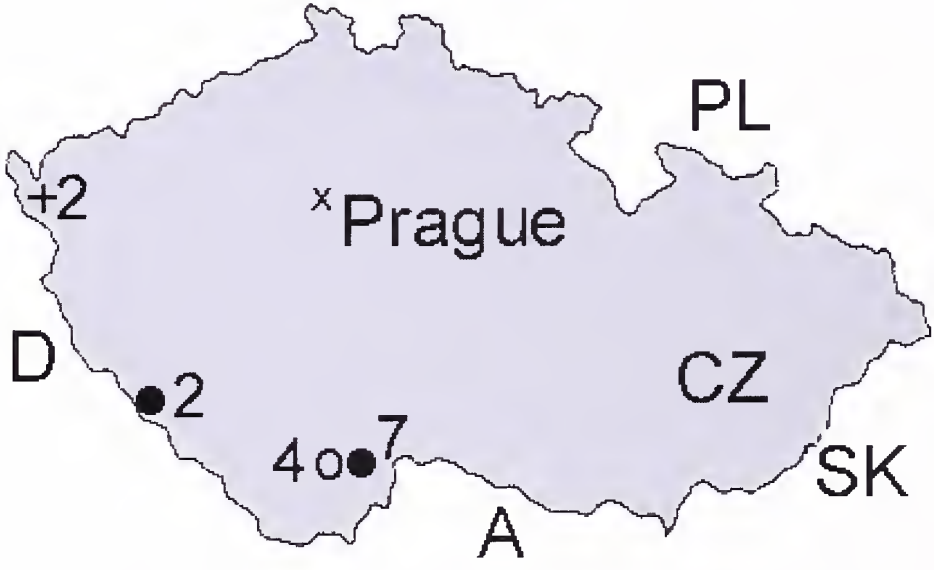


Figure 5: The map of recent (2005) natural distribution of *U. ochroleuca* s. lato (crosses), *U. ochroleuca* s. str. (empty circles), and of *U. stygia* (full circles) in the Czech Republic. For each region and species, the number denotes the number of recent natural sites.

and *U. ochroleuca* s. str. by applying the following criteria for the plants from the Třeboň Basin and/or the Czech Republic (see also Figures 2-4):

*Utricularia stygia*: the mean angle between the shorter arms is always below 85° but the maximum angle below 115°.

*Utricularia ochroleuca* s. str.: the mean angle between the shorter arms is always above 100°, the minimum angle can also be only 70° but there are always maximum angles (at least in 10% of glands) above 120° and usually above 130°.

We observed a great variability of the size parameters of the arms and angles between arms in traps of the both studied species, on the level of both within-population and between-population. It is necessary to emphasize that the same great variability of the angles between shorter arms exists also within single traps. The situation is clear in *U. stygia* traps with relatively low variation (see Tables 1, 2; Figure 2). However, as found in traps of *U. ochroleuca* s. str. there are small patches of quadrifid glands with the angles reminding *U. stygia* (see Figure 3). For a reliable determination, it is therefore necessary to investigate as many glands as possible (at least 40-50) within each trap and focus mainly on the above maximum angles. Moreover, to inspect at least five traps.

The great within-population variability could reflect a great variability of microhabitat factors (e.g., water level, oxygen concentration, temperature, prey availability, etc.), some of which could influence gland development. In our study, there was a great quantitative difference in parameters between field-collected *U. stygia* plants from Ponědrážka site and those grown in outdoor culture (Table 1). Taylor (1989) noted also a great variation in *Utricularia* quadrifid glands in the same trap, between traps on the same plant, and between plants from different populations.

According to Thor (1988), the both species also differ slightly in the color and shape of corolla. *Utricularia ochroleuca* s. str. has light yellow corolla with its lower lip almost flat or downwards deflexed margins and spur length is only about 3 mm. Whereas *U. stygia* has yellow corolla with a reddish tinge and lower lip is flat or slightly curved upwards; spur length is 4-5 mm. At some investigated sites (Hliníř and Vizír) at which we had determined *U. stygia* using the angles of the arms, we later found flowers which corresponded to the taxon of *U. stygia*. They reminded those in the Institute's culture (see Figure 1, collected from Švarcenberk) and were dark yellow with red ribs and 4-5 mm long spur.

According to Taylor (1989), *U. ochroleuca* s. lat. is not one but several morphologically slightly different clones which reproduce only vegetatively and might have different chromosome numbers. Schlosser (2003) showed that also populations of *U. ochroleuca* from North America (which he described as *U. ochroleuca* s. lat.) were very different from each other and also different from typical Scandinavian *U. ochroleuca* s. str. Summarily, the complex *U. ochroleuca-U. stygia* probably consists of sterile hybrids which have different mother plants (*U. minor* or *U. intermedia*) and originated at different time and places. Differences between Scandinavian and Central European populations of *U. ochroleuca* might be connected also with glaciations. "Nordic" *U. ochroleuca* s. str. might be a young taxon which has spread on a deglaciated area over Scandinavian Peninsula. It should be added that also Schlosser (2003) considered southern populations as evolutionary old.

*Utricularia stygia* has a wide circumboreal distribution in the northern hemisphere. According to Schlosser (2003), this species is more common than *U. ochroleuca* s. str. in Europe and probably also in North America. Our results fully agree with his suggestion. In Třeboň basin in S Bohemia, *U. stygia* has been verified recently (2005) to have seven natural sites (and one artificial) whereas *U. ochroleuca* s. str. only four sites. In the whole Czech Republic, *U. ochroleuca* s. lato has two other sites in W Bohemia and so has also *U. stygia* in SW Bohemia (see Figure 5; Adamec, unpubl.). Moreover, there is a great difference in the size of the popula-

tions of both species. While all *U. stygia* populations investigated are usually within the order of magnitude of thousands to hundreds of thousands of shoot apices those of *U. ochroleuca* s. str. have only dozens or a few hundred shoot apices (Adamec, unpubl.) and may be characterized as vanishing. However, both species are rare and threatened with extinction in the Czech Republic.

Nevertheless, *U. stygia* behaves as a stronger competitor than *U. ochroleuca* s. str. as it can be judged from the fact of natural spreading of the former species to a previous *U. intermedia* site at Ruda peat bog (Navrátilová & Adamec, unpubl.). Moreover, in contrast to *U. australis*, they both have a narrow ecological range and meet specific habitat requirements. In S Bohemia, they grow in peat bogs, usually in the littorals of eutrophic fishponds (Adamec & Lev 2002), and these shallow dystrophic and oligo-mesotrophic waters are very sensitive to eutrophication, overgrowing, and drainage. According to Thor (1988), in Nordic countries *U. stygia* grows only in oligotrophic habitats; in stagnant, shallow waters in wetlands and peat bogs, while *U. ochroleuca* s. str. grows usually in slightly eutrophic and semi-rich habitats. In S Bohemia, there is evidently no habitat segregation between the species.

Conservation measures should consist of protection of habitat and its sustainable development. In addition, the propagation of the plants ex situ and their subsequent (re)introduction to old natural and new sites within the same area are recommended. The first step has been made because both South Bohemian *U. stygia* and *U. ochroleuca* s. str. have been successfully cultivated in the outdoor Collection of Aquatic and Wetland Plants in the Institute of Botany at Třeboň. Cytological-anatomical studies on South Bohemian *U. stygia* have also been started (Płachno et al., 2005). Search for seeds and embryological study of both species, such as in *U. bremii*, could be beneficial as fertile plants producing seeds could be found in some populations (Adamec 2002).

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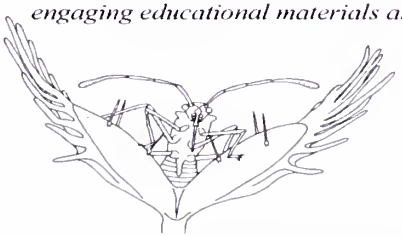

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